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Pharmaceutical Applications of Polymorphism

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Keyphrases ☐ Polymorphism—pharmaceutical applications ☐ Stability, chemical—polymorphorphism ☐ Methodology—polymorphism determination ☐ Metastable polymorphs—preparation

A polymorph is a solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of the molecules of that compound in the solid state. The molecule itself may be of different shape in the two polymorphs, but that is not necessary and, indeed, certain changes in shape (involving dynamic isomerism or tautomerism) involve formation of different molecules and hence do not constitute polymorphism. Geometrical isomers or tautomers, even though interconvertible and reversibly so, cannot be called polymorphs although they may behave in a confusingly similar manner. Shape changes, permissible in the molecule crystallizing in two or more polymorphic forms, include resonance structures, rotation of parts of the molecule about certain bonds, and minor distortions of bond distances and angles. These distortions of molecular shape result from polarizability effects of one molecule on another due to the change in relative positions of adjacent molecules in the two different crystalline arrangements.

A safe criterion for classification of a system as polymorphic is the following. Two polymorphs will be different in crystal structure but identical in the liquid and vapor states. Dynamic isomers will melt at different temperatures, as do polymorphs, but will give melts of different composition. In time each of these melts changes to an equilibrium mixture of the two isomers with temperature-dependent composition. Some reported cases of polymorphism are undoubtedly dynamic isomerism, since the two behave quite similarly, especially if the equilibrium between the two isomers is very rapidly established.

Polymorphism is the ability of any element or compound to crystallize as more than one distinct crystal species (*e.g.*, carbon as cubic diamond or hexagonal graphite). Different polymorphs of a given compound are, in general, as different in structure and properties as the crystals of two different compounds. Solubility, melting point, density, hardness, crystal shape, optical and electrical properties, vapor pressure, *etc.*, all vary with the polymorphic form. In general, it should be possible to obtain different crystal forms of a drug and thus modify the performance properties for that compound. To do so requires a knowledge of the behavior of polymorphs.

Mitscherlich (1) is generally given credit for first using the term polymorphism during his work on the isomorphous sulfates of iron (ferrous), cobalt, nickel, magnesium, copper, zinc, and manganese. It is, however, obvious that the idea was not new at that time, since Humphrey Davy in 1809 pointed out that diamond and graphite are both carbon and that the two differ only in their arrangement of carbon atoms in the solid phase. Indeed, Klaproth may have been the first to be aware of polymorphism when he observed (1788) that calcium carbonate crystallizes both as calcite and as aragonite.

Since that time a very large number of compounds, organic and inorganic, as well as the elements themselves, have been shown to crystallize in two or more different crystalline arrangements—chemically identical, physically different. Besides graphite and diamond there are, to name a few in the mineral field, wurtzite and sphalerite (ZnS); calcite, aragonite, and vaterite ($CaCO_3$); rutile, brookite, and anatase (TiO_2). Most polymorphs, especially those of organic compounds, do not have special names; instead they are referred to as α,

β, γ, *etc.*, or I, II, III, *etc.* Many compounds exist in five, six, and even ten, eleven, or more different crystal forms. Ammonium nitrate has five forms, progesterone also has five, water has eight or nine, tripalmitin has seven, and some drugs have been found to have ten or more different crystal forms. It is now apparent that most, if not all, compounds and elements show a varity of different crystal forms. Deffet (2) has summarized the properties of a number of organic systems exhibiting polymorphism.

The subject of polymorphism has also been covered in several texts including those by O'Connor (3), Hartshorne and Stuart (4), Kofler and Kofler (5), McCrone (6, 7), and Verma and Krishna (8).

The scientific literature also included numerous indications of its importance in pharmaceuticals. Several authors have systematically studied different classes of drug compounds. In Austria Kuhnert-Brandstätter *et al.*, using thermomicroscopic methods, have reported on the polymorphism of steroids (9–12), barbiturates (13–15), and antihistamines (16). Their work probably represents the most intensive study of polymorphism and drugs. Similarly, in England, Mesley *et al.*, using IR spectroscopy, have described the polymorphism of steroids (17, 18), barbiturates (19, 20), and sulfonamides (21). While the subject of polymorphism is extensively covered in the scientific literature, there are relatively fewer reports regarding its importance in the area of pharmaceutics. The following reviews the available literature and includes a discussion of how the principles of crystal chemistry can be applied to solve various dosage form problems arising from misuse or a lack of understanding of how the solid state properties of a drug substance can affect its stability and availability.

In a 1965 survey, Kuhnert-Brandstätter (22) reported that (see Table I), of 48 steroids studied with m.p.'s less than 210°, 67% exhibited polymorphism. Out of 40 sulfonamides studied, 40% exhibited polymorphism, and out of 38 barbiturates studied, 63% existed in different polymorphic forms. When checking marketed products she found that 17% of the steroids, 23% of the sulfonamides and 11% of the barbiturates were unstable, as a result of polymorphic changes in the system.

Table I•—Polymorphism of Drugs

Compd.	No. Studied	% Having Polymorphs	% of Unstable Samples
Steroids (m.p. less than 210°)	48	67	17
Sulfonamides	40	40	23
Barbiturates	38	63	11

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APPLICATIONS OF POLYMORPHISM IN THE PHARMACEUTICAL INDUSTRY

Preparation of Physically Stable Dosage Forms

Suspensions—*Aqueous Vehicles*—Due to use of a wrong polymorph of a drug, a phase conversion from the metastable to stable polymorph may occur. This produces:

(*a*) Crystal growth, resulting in undesirable particle size distribution. This can produce serious problems with parenteral suspensions where syringibility of the product can become difficult if significant particle growth occurs. Biological availabilities of the drug also can be altered because phase transitions produce drug particles having different solubilities.

(*b*) Caking, producing suspensions that cannot be uniformly resuspended by shaking. A good example of suspensions in aqueous vehicle is the cortisone acetate suspension. Cortisone acetate was one of the most difficult polymorphic problems to solve. Macek (23) obtained the first patent on stable noncaking aqueous suspension of cortisone acetate and methods of preparing the same. He described the early attempts to obtain a stable aqueous suspension, where cortisone acetate, in the form of crystals stable in the dry state, was suspended in the aqueous medium and allowed to remain in the medium for a few hours. It was observed that crystal growth of the cortisone acetate invariably occurred with subsequent caking and sedimentation. A physically stable aqueous suspension was obtained by ball-milling cortisone acetate powder, referred to by Macek as Form 2, in the aqueous vehicle where a polymorphic phase transition occurred, to Form 5. In a later patent, Magerlein (24) described two new polymorphs of cortisone acetate, Form *A*, which is not stable in the dry state, and Form *B*, which is stable in the dry state. Both crystal forms when used in aqueous suspensions gave physically stable, noncaking aqueous suspensions. The cause of the growth of crystals of Form 5, in the early suspensions prepared by Macek, is due to greater solubility of Form 2 in the solution phase than Form 5. The concentration of Form 2 is greater in the solution immediately in contact with Form 5 crystals than the solubility of the Form 5. This happens because of the dissolution of more soluble Form 2 into the solution, resulting in a supersaturated solution with respect to Form 5, the stable configuration.

Creams—When creams are prepared with the active ingredient suspended in the cream base, use of the wrong polymorph can result in a phase inversion to a more stable phase. As a consequence, crystal growth can occur in the vehicle yielding gritty, cosmetically unacceptable creams or products in which the active ingredient is unevenly distributed. During the preparation of a topical cream it is necessary to select the correct polymorph of the active ingredient, which when suspended is least susceptible to growth in the cream base.

The correct procedure is to choose the polymorphic phase which is least soluble in the cream base. When a metastable phase with high solubility is suspended in the cream base there is a high risk that nucleation of a more stable (less soluble) form will eventually occur. When this happens, the crystal size distribution in the system is altered as the more stable form gradually replaces the metastable phase. The usual consequence of this process is a substantial increase in the mean crystal size of the suspended drug in the formulation. While

crystal growth of finely suspended drug particles can occur in the absence of such transformations, serious consideration should be given to the presence of different polymorphic forms when this type of problem is encountered with a formulation. In certain instances, the use of the most stable polymorph for suspension in liquid or semisolid dosage forms may not be the best procedure. For example, phase conversion may be so slow in certain ointment bases that a more soluble metastable form may be safely used. It is entirely possible the use of a more thermodynamically energetic form of the drug may result in a more efficacious therapeutic formulation.

Solutions—One of the first considerations in formulating a solution is to determine the solubility of the drug in its vehicle. If the solubility determination is conducted using a metastable form of the drug and the concentration of the drug in the system exceeds the equilibrium solubility of a less soluble form of the drug, a thermodynamically unstable formulation results. In a sense, this is akin to emulsions which are also thermodynamically unstable systems. Some solutions that are supersaturated with respect to the stable form of the drug may remain in this state for relatively long periods of time. Chance nucleation of the stable form, however, quickly results in crystallization until equilibrium is reached with respect to this form. This is a frequent problem with sparingly water-soluble drugs, such as the steroids, and this phenomenon has been frequently encountered in these laboratories.

Flynn (25) has reported an example of this type of problem in the formulation of a parenteral solution of a drug. In this instance, determination of the water solubility of this compound indicated the drug to be adequately soluble for the concentration required in the formulation. Stability studies on the formulation quickly turned up the presence of a precipitate. An investigation of the problem showed the precipitate to consist of a less soluble polymorph of the compound. The problem was solved by formulating the product in a vehicle containing sufficient cosolvent to solubilize the less soluble polymorphic form.

Suppositories—The polymorphic changes of a suppository base could result in a product that undergoes a change in its melting characteristics. If the suppository base is of the type that depends on melting at body temperatures to release the active components of the formulations a relatively small change in its melting point could have severe consequences. If the melting point is depressed the product may melt or soften at shelf temperatures. If the melting point becomes greater than anticipated, the suppository may not melt properly when administered. This point could be demonstrated by extemporaneous preparations of suppositories containing theobroma oil as their base. Theobroma oil, like many triglycerides, exhibits polymorphism (26). It exists in three different crystal forms each with different melting points. Suppositories are prepared by fusion of theobroma oil, when they are melted and brought to 60–70°. The melt is then poured into molds and quickly chilled in a refrigerator. If suppositories prepared by this method are removed from the refrigerator after a short time they will melt at 30°, which makes their use impractical in the summer, and the patients will have difficulty in inserting them, since they liquefy in the fingers. If the suppositories are prepared by heating the theobroma oil just a few degrees above its melting point, the suppositories will have a higher melting point and can be easily handled. This method of manufacture permits the crystallization nuclei of the more stable (higher melting point) β-forms to remain in the melt, which on chilling favors additional crystallization of the β-form. Fused theobroma oil heated to 60–70° when chilled undergoes supercooling and α-form crystals develop, which have lower melting points. The α-form, being a metastable phase, slowly changes to β' and to β-form.

Polymorphism and Chemical Stability

There have been a number of instances where different crystalline phases of the same compound have different chemical stabilities. One of the authors has observed this while working with aqueous suspensions of an experimental corticosteroid, when chemical instability developed in some of the batches of this compound. The raw starting material batches were checked with X-ray diffraction and the presence of two different polymorphs was confirmed. When these polymorphs were further studied for their chemical stability it was found that one of these was light-sensitive. Batches containing this crystal form then decomposed with time and assayed lower than the other batches. This chemical sensitivity could be due to solvents occluded or absorbed mother liquor, where the latter could effect chemical stability, or in cases of polymorphs due to different light absorption patterns. The patterns would differ slightly and one must absorb a frequency that causes a photochemical decomposition. Similarly, Macek (27) has reported that the amorphous forms of the sodium and potassium salts of penicillin G obtained by evaporation from solution, are less stable chemically than their crystalline counterparts. For example, crystalline potassium penicillin can withstand dry heat for several hours without significant decomposition. Under similar conditions, the amorphous forms lose considerable activity. "This property is important if one is interested in depositing penicillin on a solid, as in tablet coating. Application from solution in a volatile solvent obviously would lead to greater instability, whereas deposition of a suspension of the crystalline form, even though in a very fine state of subdivision, would be expected to result in greater stability" (27).

In such cases where chemical stability is a problem, there obviously is a need for careful control during chemical manufacture to assure that the desired polymorphic form is obtained.

Polymorphism and Generically Equivalent Dosage Forms

If the rate of absorption of the active ingredient in an oral preparation is dissolution-rate dependent, the use of a compound exhibiting polymorphism may lead to good or bad consequences. The successful utilization of a polymorph of significantly greater thermodynamic activity (*i.e.*, solubility) than the stable modification may provide, in some instances, therapeutic blood

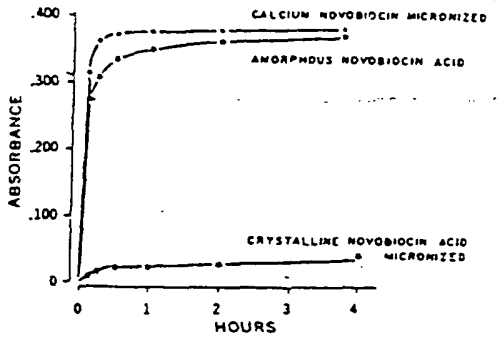

Figure 1—*Absorbance of novobiocin in 0.1 N HCl at 305 mμ.*

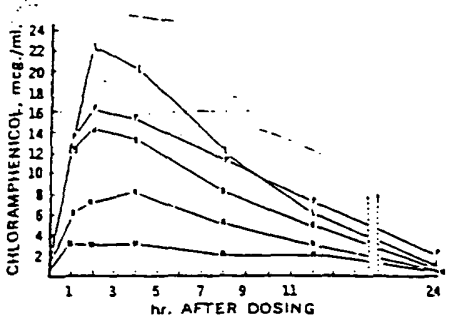

Figure 2—*Comparison of mean blood serum levels obtained with chloramphenicol palmitate suspensions containing varying ratios of A and B polymorphs, following single oral dose equivalent to 1.5 g. chloramphenicol. (Percent polymorph B in the suspension: M, 0%; N, 25%; O, 50%; P, 75%; L, 100%.)*

levels from otherwise inactive drugs. On the other hand, when the existance of multiple crystalline modifications goes unrecognized in a particular formulation, this may possibly result in unacceptable dose-to-dose variations in drug availability to the patient (28).

Mullins and Macek (29) working on pharmaceutical properties of novobiocin identified two forms of novobiocin, one of which is crystalline and the other amorphous. In tablet and capsule formulations novobiocin is used as the sodium salt which is active orally but it is unstable chemically in a solution, while the insoluble forms of novobiocin acid are more stable chemically. But the crystalline novobiocin acid is poorly absorbed and does not provide therapeutically adequate systemic levels following oral administration. The amorphous acid is readily absorbed and is therapeutically active. This difference in availability is due to differences in solubility in aqueous systems. When an excess of crystalline or amorphous novobiocin acid in less than 10-μ size were shaken in 0.1 *N* hydrochloric acid at 25°, the amorphous solids were at least 10 times more soluble than the crystalline acid (see Fig. 1). This difference in solubility might be expected to favor the absorption of the amorphous solid from the gastrointestinal tract. Data showing differences in novobiocin plasma levels in drug following oral administration of 12.5 mg./kg. each of amorphous novobiocin and crystalline novobiocin acid and the sodium salt are shown in Table II.

Unless special precautions are taken to maintain the solid in suspension in amorphous state by the addition of materials to suppress crystallization, amorphous novobiocin converts slowly to a crystalline form. The formulation becomes less and less absorbable and finally loses therapeutic effect entirely. To stabilize the suspensions they conducted a search for additives that would significantly retard or even prevent crystallization of aqueous suspensions of amorphous novobiocin, and they found that some agents provided adequate protection against crystallization for significant periods of time. The best agents found were methyl cellulose, PVP, and several alginic acid derivatives such as sodium alginate and propylene glycol algin.

Chloramphenicol palmitate exists in four polymorphs, three crystalline (30) (*A*, *B*, and *C*), and an amorphous one (31, 32). Aguiar *et al.* (30) investigated the absorption of Polymorphs *A* and *B* to determine the effect of varying concentrations (percent of Polymorph *B* was 0, 25, 50, 75, and 100). After oral ingestion of the suspension (equivalent to 1.5 g. chloramphenicol) blood and urine specimens were collected for a 24-hr. period. The mean blood levels obtained are shown in Fig. 2 and the urinary excretion data are given in Fig. 3. In these single dose studies the highest mean blood levels were obtained with suspensions containing only Form *B*. The blood levels decreased proportionately as the concentration of Form *A* increased. These data demonstrate that absorption is influenced by the type and concentration of the crystal polymorph present.

Table II—Novobiocin Plasma Levels in Dogs Following Oral Administration of Different Solid Forms*

Hours after Dose	Sodium Novobiocin, mcg./ml. Plasma	Amorphous Novobiocin (Acid), mcg./ml. Plasma	Crystalline Novobiocin (Acid)
0.5	0.5	5.0	N.D.†
1	0.5	40.6	N.D.†
2	14.6	29.5	N.D.†
3	22.2	22.3	N.D.†
4	16.9	23.7	N.D.†
5	10.4	20.2	N.D.†
6	6.4	17.5	N.D.†

* Dose = 12.5 mg./kg. † Not detectable.

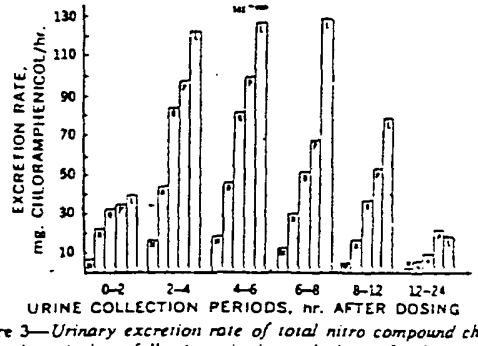

Figure 3—*Urinary excretion rate of total nitro compound chloramphenicol equivalent following single oral dose of chloramphenicol palmitate suspensions containing varying quantities of Polymorphs A and B. Dose equivalent to 1.5 g. of chloramphenicol. (Percent Polymorph B in the suspension: M, 0%; N, 25%; O, 50%; P, 75%; L, 100%.)*

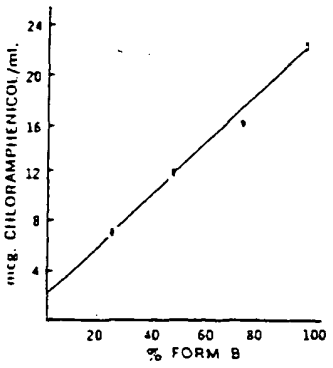

Figure 4—*Correlation of "peak" blood serum levels (2 hr.) vs. percent concentration of Polymorph* B.

The differences in absorption are even more evident in Fig. 4 where the absorption at 2 hr. ("peak" blood levels) is plotted *versus* the percent of Form *B* present in the suspension. A linear relationship apparently exists between the peak levels and concentration of Polymorph *B*. The blood levels increase in a direct relationship to the increase of Polymorph *B*. One year before Aguiar's paper, Anderson (33) of the National Biological Standards Laboratory of Canberra, Australia, investigated a complaint that a chloramphenicol suspension had had an unsatisfactory therapeutic effect. Several commerical preparations and powders of chloramphenicol palmitate were examined. Of six powders, four appeared to consist mainly of the Polymorph *A*, the inactive type, and of seven samples of suspensions, one contained mainly inactive Polymorph *A*.

Poole *et al.* (34) reported on physiochemical factors influencing the absorption of the anhydrous and trihydrate forms of ampicillin. They found that aqueous solubility of the anhydrous form was 20% higher than the trihydrate at 37° (10 and 8 mg./ml., respectively, for the anhydrous and trihydrate forms). They also determined the effect of the observed solubility differences on the *in vitro* availability of the drug. In the *in vitro* experiments they measured the T_{50} (time required for 50% of

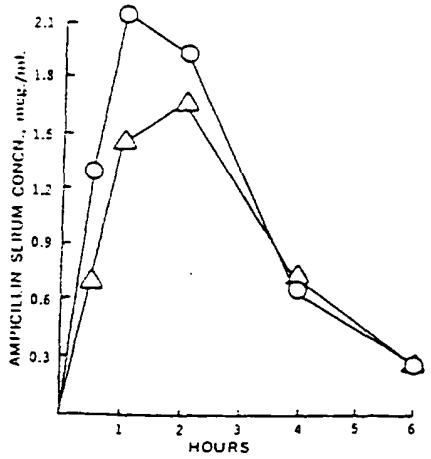

Figure 5—*Mean blood serum concentrations of ampicillin in human subjects after oral administration of 250-mg. doses of the oral suspension. Key:* ○, *anhydrous;* △, *trihydrate [reproduced with permission from Current Therap. Res., 10, 299(1968)].*

Table III—Area Under the Blood Serum Level–Time Curve Observed in Human Studies*

Formulation	Form of Ampicillin	Area, cm.²	Ratio, Anhydrous-/Trihydrate
Suspension	Anhydrous	143.5	1.21
	Trihydrate	119.0	
Capsules	Anhydrous	127.8	1.17
	Trihydrate	109.0	

* Reproduced with permission from *Current Therap. Res.*, 10, 302 (1968).

the labeled amount to appear in solution) and they found that it was 7.5 and 45 min for the anhydrous and trihydrate forms, respectively. They also designed experiments to correlate the *in vitro* data with *in vivo* drug availability. Their *in vivo* experiments were done with dogs and human subjects, where the anhydrous and the trihydrate form of the drug were given as oral suspensions or capsules. The anhydrous form produced higher and earlier peaks in the blood serum than the trihydrate form. This was more pronounced in the suspension formulations. Figure 5 shows the mean blood serum concentration in human subjects after oral administration of a 250-mg. dose of the suspension. With both formulations the area under the blood serum level *versus* time curve was greater with the anhydrous form (see Table III), indicating that the anhydrous form is more efficiently absorbed.

Hamlin *et al.* (35), using two polymorphs of methylprednisolone (Forms I and II), prepared constant surface pellets and determined their dissolution rates by four different *in vitro* methods. These results were compared with *in vivo* dissolution rates obtained by implanting pellets in rats according to the method of Ballard and Nelson (36). They found that (see Table IV) *in vivo* the dissolution rate of Form II was 1.2 times greater than Form I, the thermodynamically more stable form at room temperature. While in the *in vitro* studies where the agitation intensity was of a low-order Form II had a dissolution rate 1.53 times more than Form I when studied with the hanging pellet method (37), and when studied by pellet holder method in the Wruble machine (38) at 6 r.p.m. the dissolution of Form II was 1.3 times more than Form I. At higher agitation intensities these differences disappeared. In a similar study, Ballard and

Table IV—Results of Dissolution Rate Experiments Performed with Constant Surface Pellets of Methylprednisolone Polymorphs I and II by an *in vivo* and Several *in vitro* Methods

Test	Methylprednisolone Polymorph	Dissolution Rate, mg./cm.²/hr.
Pellet implant in rats	I	0.0179
	II	0.0302
Hanging pellet method	I	0.091
	II	0.139
Pellet holder method in Wruble machine, 6 r.p.m.	I	0.203
	II	0.265
Pellet holder method in Wruble machine, 12 r.p.m.	I	0.276
	II	0.275
Pellet holder method in machine of Souder and Ellenbogen (39), 40 r.p.m.	I	0.630
	II	0.656

Table V—Absorption Rate of Hydrocortisone TBA and Prednisolone TBA

Crystal Modification	Absorption Rate per Unit Area (mg./hr./cm.^{2})
Prednisolone TBA	
Phase I (anhydrous phase)	1.84×10^{-3}
Phase II (monoethanol solvate)	8.70×10^{-3}
Phase IV (hemiacetone solvate)	2.20×10^{-3}
Hydrocortisone TBA	
Phase I (monoethanol solvate)	1.83×10^{-3}
Phase II (monoethanol solvate)	7.32×10^{-3}
Phase III (hemichloroform solvate)	7.40×10^{-3}
Phase IV (anhydrous)	4.74×10^{-3}

Biles (40) studied the *in vivo* absorption rates of hydrocortisone *tert*-butyl acetate and prednisolone *tert*-butyl acetate and their solvates by the pellet implantation technique. They found that solvates affect the solid-drug absorption rate (see Table V). With prednisolone *tert*-butyl acetate pellets, for example, the monoethanol solvate (Phase II), had 4.7 times the mean absorption rate per unit area of anhydrous phase (Phase I). The hemiacetone solvate (Phase IV) had practically the same mean absorption rate as the anhydrous phase. With hydrocortisone *tert*-butyl acetate pellets, the mean absorption rate for the solvates were all significantly different from the mean rate of the anhydrous phase (Phase IV). Also, one of the monoethanol solvates (Phase II) had 4.0 times the mean absorption rate of the other monoethanol solvate (Phase I), these results indicate that drug solvates may exhibit dimorphism and each form may exhibit different *in vivo* absorption rates. In a comparison of the physical properties and biological activities of some crystalline phases of fluprednisolone, Haleblian and Biles (41) isolated one amorphous and six crystalline phases. Two phases were dimorphic clathrates containing 1 mole of water. One existed as the *tert*-butylamine dissolvate and three were anhydrous trimorphs. Using the Wruble (38) apparatus they determined the *in vitro* aqueous dissolution rates (6 r.p.m. and 23°) of the anhydrous and hydrated forms as seen in Fig. 6. It was found that the dissolution rates ratio for the highest and lowest energetic phases was 2.24 where the Form I was the most energetic phase and the α-monohydrate the least energetic phase.

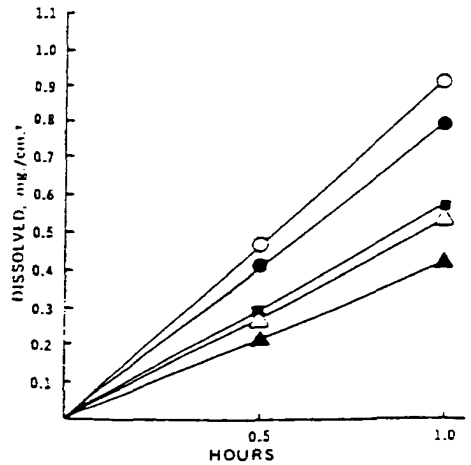

Figure 6—*Dissolution rates of the various phases of fluprednisolone in distilled water at 23°C. and 6 r.p.m. Key:* ○, *Form I;* ●, *Form III;* ■, *Form II;* △, *β-monohydrate;* ▲, *α-monohydrate.*

Similar data were obtained by comparing the *in vivo* dissolution rates of the pellets of each crystalline phase which had been implanted subcutaneously by the method of Ballard and Nelson (36) into male rats.

The *in vivo* dissolution rates of different phases of fluprednisolone are presented in Table VI. The *in vivo* dissolution-rates ratio between the highest and lowest energetic phases was found to be 1.61. Thus, the biological uptake of Form I of fluprednisolone in pellet form was 1.61 times that of the α-monohydrate phase. This is an illustration of the effect that crystalline phases have on biological activity. The *in vivo* and *in vitro* dissolution rates were also correlated with a pharmacologic response—adrenal cortex atrophy of the rats. The adrenal cortex atrophy resulting from the uptake of Form I from pellet implants was 1.46 times that of α-monohydrate. Table VII compares the effect of different polymorphs and hydrates on *in vitro* and *in vivo* dissolution rates and adrenal cortex atrophy. These results suggest that care must be taken in the use of raw material when polymorphism is a physical characteristic of a particular compound.

In formulating injectable insulin the duration of the action is controlled by its crystallinity. Insulin precipitates as an insoluble complex when reacted with zinc chloride and, depending on the pH, it may precipitate either as an amorphous or crystalline phase. Prompt insulin zinc suspension USP (42) consists of amorphous insulin zinc complex. It is readily absorbed when injected and has a relatively short duration of action. Extended insulin zinc suspension USP (42) is made up of crystalline zinc complex. It is very slowly absorbed and has a longer duration of action. Insulin zinc suspension USP (42) is made up of a mixture containing seven parts of crystalline and three parts of amorphous insulin zinc complex and it is intermediate in duration of action. Another difference in the formulations is their particle sizes; prompt insulin is made up of small particles and extended insulin is made up of large particles. This is another example where the rate of absorption and duration of action is determined both by particle size and crystal form.

Levy (43) has reported on the comparative dissolution and absorption rates of different commercial aspirin tablets and indicated that since the initial absorption of aspirin occurs from the stomach, and since the rate of absorption is proportional to the amount of aspirin

Table VI—*In Vivo* Dissolution Rate of Fluprednisolone Implants

Phase	Dissolution Rate, mg./cm.^{2}/hr.	Ratio[a]
Form I	0.237	1.61
Form III	0.209	1.42
Form II	0.186	1.26
β-Monohydrate	0.162[b]	1.10
α-Monohydrate	0.147[b]	1.00

[a] Compared to α-monohydrate. [b] Corrected for *in vivo* dissolution rate of anhydrous fluprednisolone.

Table VII—Comparison of *In Vitro* and *In Vivo* Dissolution Rates and Adrenal Cortex Atrophy

Phase	*In Vitro* Dissolution Rate at 23°C. and 6 r.p.m. (mg./cm.²/hr.)	Ratio[a]	*In Vivo* Dissolution Rate (mg./cm.²/hr.)	Ratio	Adrenal Cortex Atrophy (g. Atrophy/g. of Rat wt./hr. × 10^{-7})	Ratio
Form I	0.917	2.24	0.237	1.61	8.05	1.46
Form III	0.804	1.96	0.209	1.42	7.09	1.28
Form II	0.571	1.39	0.186	1.26	6.57	1.19
β-Monohydrate	0.527	1.29	0.162[b]	1.10	6.11	1.11
α-Monohydrate	0.410	1.00	0.147[b]	1.00	5.52	1.00

[a] Compared to α-monohydrate. [b] Corrected for *in vivo* dissolution rate of anhydrous fluprednisolone.

dissolved in the gastric fluids, the *in vivo* dissolution rate of aspirin in tablet form in the stomach would be reflected. It is possible that polymorphism may also be involved with availability from commercial aspirin tablets. Tawashi (44) has reported on the dissolution of two polymorphic forms of aspirin where Form II dissolves 50% faster than Form I. Ballard and Nelson (36) have investigated the absorption rate of pellets after subcutaneous implantation. Their absorption data on anhydrous tetracycline pellets were anomalous since the mean weight after implantation was greater than before. They observed, "The increase in mean weight could not be explained on the basis of 'ghost' formation. The increase in weight could be satisfactorily explained assuming that the anhydrous tetracycline was converted to the trihydrate in the body. Thus, the weight loss of the pellets due to absorption was more than compensated for by the increase in molecular weight due to hydration."

Polymorphism and Tableting Behavior of Powders

Shell (45) described the use of different habits of the same compound and their effects on tableting behavior. (The outer appearance of a crystal is its habit while polymorphism is a function of the internal structure of crystals.)[1] Shell found that the ease or difficulty of tableting a powder where the active ingredient makes up a large portion is due mostly to "anisotropy of cohesion and of hardness which is possessed by organic crystals and, therefore, of most pharmaceutically important compounds" (45).

According to Shell (47), polymorphs of the same compound, which can crystallize in different habits, when forming a large portion of the tableting mixture, can exhibit similar problems. The choice of the right polymorph, all other conditions being equal, will be the one with a habit which can be tableted easily.

Miscellaneous Applications of Polymorphism

One other potential application of polymorphism which could be used in the pharmaceutical industry is preparation of fine particles, about micron size, by using the density difference for enantiotropic polymorphs.

Different polymorphs of the same compound have different densities. Due to this phenomenon, when one polymorph is heated above its conversion temperature to another polymorph, and then cooled to room temperature, strains can develop in the crystal and produce fracturing into finer particles. This type of operation would require existence of suitable polymorphic forms and that the repeated temperature cycling would not produce chemical degradation. This mechanism of particle size reduction, in certain instances, might prove to be more efficient than present methods of micronization.

Hsiachen and Grabar (48) reported on a novel effect of polymorphism in solid-state polymerization. They found that tributylvinylphosphonium bromide exists in three phases. Of these, Phase II is a metastable phase, and it polymerizes faster than the other phases, which is due to steric and collision factors which are governed by crystalline structure.

Methods Used to Study Polymorphism

A number of techniques have been used to identify different polymorphic phases of a compound. Each of these techniques could be successful in identifying the phase, but a combination of methods provide a powerful means for identification and isolation of each crystalline modification (49).

Microscopy—*Optical Crystallography*—Biles (50) in his review of crystallography has discussed optical crystallography and its application to identification of polymorphs. Different polymorphs of a crystal may belong to one of two classes depending on the effect of the transmission of light in different directions through the crystals. These are isotropic and anisotropic classes. In the isotropic crystals the velocity of light, or the refractive index which depends on the velocity of light, is the same in all directions, and in the anisotropic crystals there may be two or three different light velocities or refractive indices. Different polymorphs having dif-

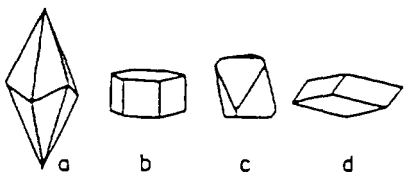

Figure 7—*Crystal habits of calcite.*

[1] A good example in differentiating polymorphism and habit is $CaCO_3$. Calcite, one of the polymorphs of $CaCO_3$, crystallizes in the trigonal system and shows 4 different habits as seen in Fig. 7 (46), where each of these habits belong to the same crystal class and are developed upon the same internal structure. While aragonite, the other polymorph of $CaCO_3$ is orthorhombic and does not have the same internal structure.

ferent internal structures will belong to different crystal systems and have different sets of refractive indices. Biles (49) reported the optical crystallographic properties of some polymorphs of prednisolone and hydrocortisone *tert*-butyl acetate, while Trivedi *et al.* (51) reported the optical crystallographic properties of ouabain hydrates. Eisenberg (52) compiled the optical crystallographic characteristics of some NF drugs exhibiting polymorphism.

Hot Stage Methods—The polarizing microscope fitted with a hot stage (or cold stage) is a very useful tool for investigating polymorphism. With this combination an experienced microscopist can quickly tell (*a*) whether polymorphism exists; (*b*) the degree of stability of the metastable forms; (*c*) transition temperatures and melting points; (*d*) rates of transition under all temperature and physical conditions; (*e*) whether to pursue polymorphism as a route to an improved dosage form. These methods are discussed in detail by the Koflers (5) and by McCrone (6, 53).

X-Ray Powder Diffraction—Crystalline materials in powder form give characteristic X-ray diffraction patterns made up of peaks in certain positions and varying intensities. From the 2θ values of these peaks, the spacing values (d distance) for the different planes of the crystal can be calculated using the Bragg equation, $n\lambda = 2d \sin \theta$, where the wavelength of the X-ray source is known. Each powder pattern of the crystal lattice is characteristic for a given polymorph. X-ray powder diffraction has the advantage over other identification techniques in that the sample is examined as presented (after size reduction), very small amounts of samples are needed, and the sample can be recovered since the method is nondestructive. Since the diffraction peaks are additive for mixtures of compounds care must be taken to insure that the samples do not contain impurities. X-ray powder diffraction is one of the most widely used techniques after optical microscopy. Several investigations have used this method to identify polymorphs of pharmaceuticals (20, 49, 51, 54–56).

Infrared Spectroscopy—In identification of different polymorphs with IR spectroscopy only solid samples (as mineral oil mulls or potassium bromide pellets) can be used, since in solution polymorphs of a compound have identical spectra. Many authors (18, 20, 54, 57, 58) have used IR spectroscopy to study polymorphism. Kendall (57) claimed that, in addition to being rapid, the technique is both quantitative and qualitative. Smakula *et al* (59) reported that when different polymorphs of estradiol-17β were triturated as a mull for different time intervals the IR absorption spectra for these phases were changed to a common spectrum.

Differential Thermal Analysis—In differential thermal analysis (DTA), heat loss or gain resulting from physical or chemical changes occurring in a sample is recorded as a function of temperature as the substance is heated at a uniform rate. Enthalpic changes, both exo- and endothermic, are caused by phase transitions. For example fusion, boiling, sublimation, vaporization, crystalline structure inversion, solid-solid transition, and water loss generally produce endothermic effects,

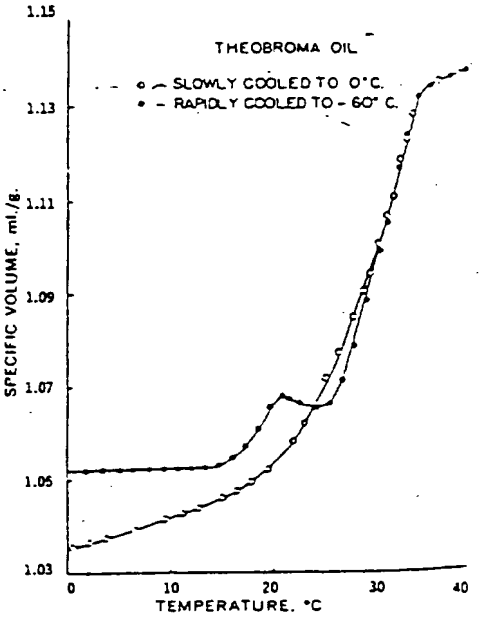

Figure 8—*Dilatometric curves, theobroma oil, slowly and rapidly cooled.*

whereas crystallization produces exothermic effects (60). One of the advantages of DTA is the ability to calculate the heats of transition from one polymorph to the other. Guillory (61) using DTA, obtained the heats of transition of methyl prednisolone and sulfathiazole polymorphs.

Dilatometry—Dilatometry is the measurement of changes of volume caused by thermal or chemical effects. Ravin and Higuchi (62), using dilatometry, followed the melting behavior of theobroma oil by measuring the specific volume of both rapidly and slowly cooled theobroma oil as a function of bath temperature (Fig. 8). The sample which was slowly cooled exhibited no unusual behavior, while the sample rapidly cooled showed an expansion behavior between 16 and 20° followed by a contraction between 20 and 24°. The authors suggested that this was probably due to an unstable modification, and the expansion followed by contraction resulted from the phase conversion from a less dense unstable form to a more dense stable form.

Proton Magnetic Resonance Spectroscopy—Chapman *et al.* (55) working with water-soluble compounds such as cephaloridine found that the combination of solid-state IR with proton magnetic resonance (PMR) measurements on heavy water solutions provided a test for polymorphism. In this method, the crystalline form is distinguished by solid state infrared and the chemical identity by PMR measurements on heavy water solutions. The PMR measurements not only confirm the structure but also yield quantitative information on solvent and other impurities, which could be very helpful in establishing the number of moles of solvent solvated with the compound under study.

Nuclear Magnetic Resonance Spectroscopy—Rudman and Post (63) investigated the NMR spectrum of cyclooctanone over the temperature range −120 to +25° and

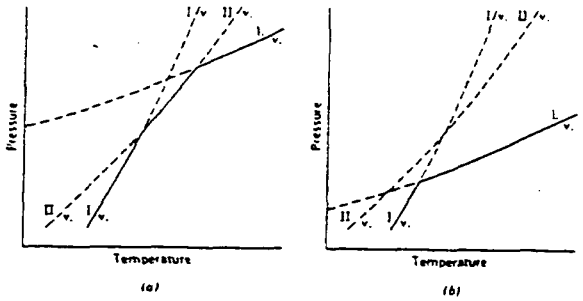

Figure 9—*Sublimation and boiling point curves for* (a) *enantiotropic system and* (b) *monotropic system.*

found that cyclooctanone forms three distinct crystalline phases in the temperature range investigated. The results were also verified by X-ray diffraction and differential thermal analysis.

Electron Microscopy—Hamm and Norman (64) in their work with organic pigments observed that both copper phthalocyanine and indanthrene blue RS can exist as crystals of varying shades. These shade differences are the result of differences in the light absorption exhibited by the polymorphic phases. They reported that the polymorphic transformations can be readily observed to take place in the electron microscope. It was found that the new forms of both pigments, completely stable to the illuminating beam after the transformation, can be seen to grow from the vapor state at the expense of the original metastable material.

The polymorphic transformations, especially in colored compounds, are almost invariably accompanied by changes in color. This is expected because the true "body" color of a solid is determined by its crystal structure as well as by its chemical chromophoric groups.

Magnetic Anisotropy—Cini *et al.* (65) identified different polymorphs of NH_4NO_3, KNO_3, $TlNO_3$, and $AgNO_3$ by following, at different temperatures, the variation of magnetic anisotropy of a powdered sample contained in a spherical container which is suspended by a torsion wire in a uniform magnetic field.

As the temperature is raised a sharp break of magnetic anisotropy occurs at the transition point of each compound (NH_4NO_3, 35, 86, and 127°; KNO_3, 127.5°; $TlNO_3$, 77.5 and 147°; and $AgNO_3$, 160°).

Study of Polymorphism

The polarizing microscope is, by far, the favored tool for the study of polymorphism (7, 53, 66-68). X-ray diffraction is also useful but the others listed are most useful for routine quality control, *e.g.*, DTA, or for elucidation of molecular differences between polymorphs, *e.g.*, NMR. Most of the rest of this paper will deal with microscopical methods.

The complete characterization of a compound should include a complete phase diagram, preferably plotted as a solubility-temperature diagram, and composition diagrams for all solid phases of the drug with all other components of the formulation. Some of the questions the investigator must be able to answer include:

1. How many polymorphic forms exist?
2. How stable are the metastable forms, and what are the relative degrees of stability for all of the polymorphic forms?
3. Is there a noncrystalline glass state and is it stable enough to consider as a dosage form?
4. Can any metastable forms be stabilized?
5. What are the temperature stability ranges for each crystal form?
6. What are the solubilities of each form?
7. How can pure and stable crystals of each form be prepared?
8. Will the more soluble metastable form survive processing, *e.g.*, micronizing or tableting?
9. Does the drug react with any other chemical component during processing or final formulation to form a molecular addition compound?
10. If so, what are its physical properties, *e.g.*, stability, solubility, and melting point, and can it exist in a desirable metastable polymorphic form or glass?

Phase Diagram—Before suggesting ways of answering some of the practical questions regarding polymorphism, it is worthwhile to review the types of phase diagrams shown by systems involving polymorphs (7). This will be done first for a simple example of a system of two forms only. There will be only one liquid-vapor (boiling point) curve in the pressure-temperature diagram, since both polymorphs give identical liquid phases on melting. Each polymorph, moreover, has its own solid-vapor (sublimation or vapor pressure) curve (Fig. 9) and its own solid-melt (melting point) curve. The complete diagram, or course, contains the melt-vapor, the solid-vapor, and the solid-melt curves (Fig. 10), and these can intersect to give either of two general possibilities, the melt-vapor curve may intersect the two solid-vapor curves above or below their intersection. It is not unknown for the three curves to intersect at the same point. When this occurs, the melting points of the two polymorphic forms and the transi-

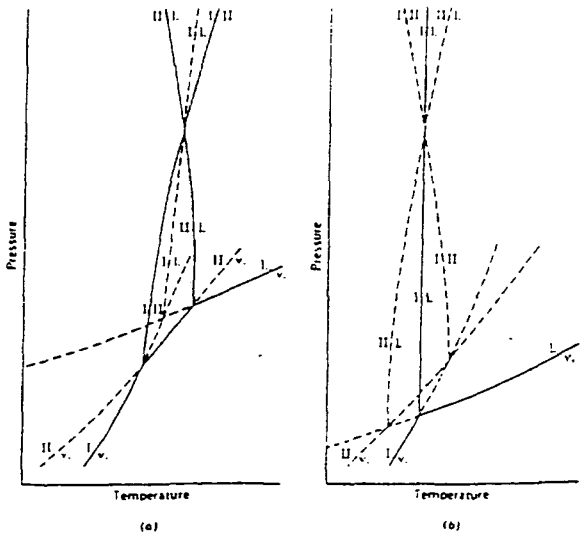

Figure 10—*Melting point and transition temperature curves added to the curves in Fig. 9:* (a) *enantiotropic* (b) *monotropic.*

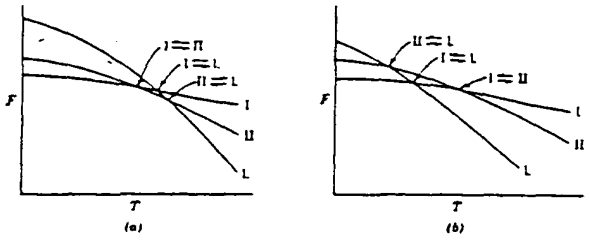

Figure 11—*Free energy-temperature curves for* (a) *enantiotropic and* (b) *monotropic systems.*

tion temperature coincide at that pressure (*e.g.*, for β-naphthol and very nearly for γ-hexachlorocyclohexane, two of whose forms melt only 0.1° apart). Figure 10 shows another way that the two melting points and the transition temperature may coincide, *i.e.*, at higher pressures where the transition curve and melting point curves coincide.

Enantiotropic and Monotropic Systems—It is not necessarily true that the melting point and transition curves intersect at high pressures, but the possibility exists. It is interesting to note that in this case the enantiotropic system becomes a monotropic system and vice versa. Considering this situation and the general fact that most systems do not show an easily determined transition temperature, it becomes obvious that these two terms, enantiotropic and monotropic, are dangerous words indeed. In the first place, the term enantiotropic can be used only when the transition temperature has been found to be below the melting point. The converse, no transition temperature below the melting point, cannot usually be interpreted to mean that the system is monotropic, because the transition temperature may be below room temperature (or below the lowest temperature studied), or it may have been unobserved because of slow transition. There is no absolutely safe generalization relating enantiotropy and monotropy to the properties of the polymorphs, except location of the transition temperature. This is best done by direct observation, but it may be done indirectly by measuring vapor pressure or solubility curves on the two forms since both sets of curves cross at the transition temperature. The form stable at lower temperatures often has the higher density, and the form stable at the melting point always melts higher and has a lower solubility and a lower vapor pressure at that temperature.

Free energy-temperature diagrams at constant pressure are another, and perhaps clearer, way of showing phase diagrams, since the phase having the lowest free energy at a given temperatue is always the stable phase at that temperature. The diagrams are based on the thermodynamic relationsbip $(dF/dT)_P = -S$. The free energy F plotted against the temperature T at constant pressure P gives a curve for each phase, the slope of which at any temperature will be the entropy S. Figure 11 shows hypothetical phase diagrams for enantiotropic and monotropic systems.

The diagrams for systems having three or more polymorphic forms can be plotted in the same way. Figures 12 and 13 show schematic P-T and F-T diagrams for HMX (cyclotetramethylene tetranitramine). Both curves are based on measured transition temperatures at atmospheric pressure. Except for these points the curves are schematic and only qualitatively correct.

Conventions for Naming Polymorphs—On the matter of convention, the various polymorphic forms are best designated by Roman numerals: I, II, III, IV, *etc.* Form I should be the form most stable at room temperature. No rigid convention can be laid down for use of the higher numerals, since further work is always attended by the possibility of discovering an intermediate Form (2.5) difficult to designate by Roman numerals and to insert without disrupting the previous assignments of numerals. A system, as logical as it is simple, is to number the forms in the order of their discovery, which should, in general, follow their order of stability. Basing the assignment on melting points is not generally satisfactory, since these data are not always available and, in many systems, cannot be determined. The Kofler suggestion that the Roman numeral be succeeded by the melting point in parentheses is a logical move subject to the preceding limitation.

Determining the Melting Points of Metastable Poly-

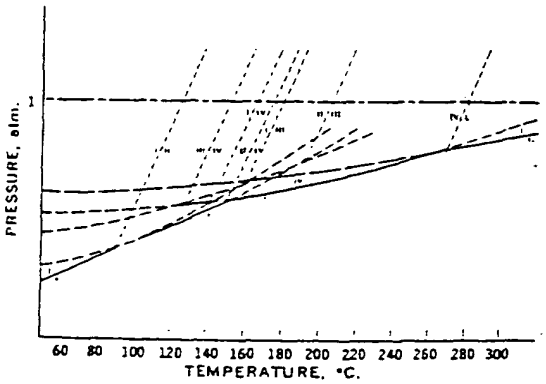

Figure 12—*Pressure-temperature diagram for HMX. The diagram is only qualitatively correct except for the intersections on the 1-atm. pressure line, which are measured points.*

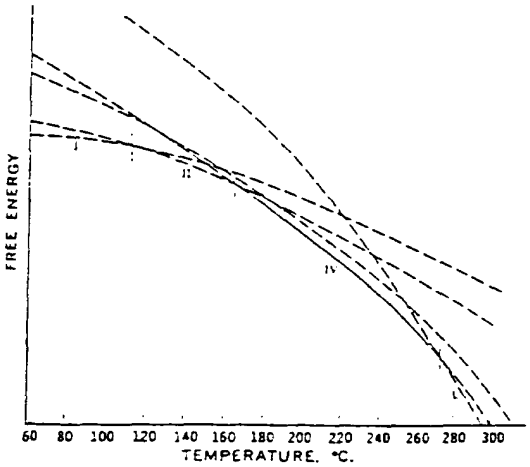

Figure 13—*Free energy-temperature diagram for HMX. The intersection temperatures are measured points, but the actual slopes are unknown.*

morphs—One of the obvious problems in studying polymorphism is the determination of the melting point for a metastable polymorph (70). This is not only necessary to characterize the system but may also be necessary to tell which form is stable (higher melting); the difference between the melting points is a measure of relative stability. If two polymorphs melt less than 1° apart, then neither is significantly more nor less stable and either may be easily obtained on crystallization. If the two melt 25 to 50° apart, the lower-melting form will be very difficult to crystallize and, once crystallized, will easily transform to the stable phase.

The closer the two melting points, the more easily the unstable form can be obtained, and its melting point can usually be obtained easily before a solid-solid transformation occurs. The chance of a solid-solid transformation can be considerably reduced, however, by using very small samples. Individual small crystals of even highly unstable forms can often be melted. Such preparations can often be produced by sublimation with a cold condensing surface. When all else fails, and the melting point cannot be determined directly, it may be possible to calculate it by using the Le Chatelier equation as suggested by Verstraete (69).

Binary Systems Involving Polymorphism—Polymorphs often show up in binary systems, and systems involving polymorphs are often more easily studied as part of a binary mixture even if this involves a solvent as a second component. Again, the microscope is an excellent way to study polymorphism in binary systems because, on a small scale, many highly unstable polymorphs are sufficiently metastable to permit measurement of melting points, transition temperatures, and concentrations. The standard microscopical physical chemical procedures are good for composition diagrams in which thermodynamic equilibrium is established, but the study of polymorphism often depends on purposely avoiding thermodynamic equilibrium or, at least, studying the rate at which equilibrium is established. With the microscope, it is possible, in most systems, to determine a complete composition diagram not only between the stable phases but also between all possible pairs of stable and metastable polymorphic forms. Figure 14 shows the composition diagram for two isomers of hexachlorocyclohexane (70), the α- and δ-isomers. The δ-isomer has two easily obtained polymorphs, and the two melting

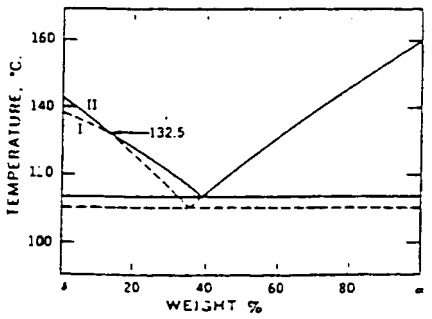

Figure 14—*Composition diagram for α- and δ-hexachlorocyclohexane showing the relationship between the two polymorphs of the δ isomer and α.*

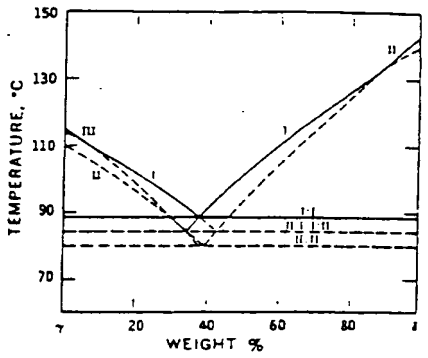

Figure 15—*Composition diagram for the three polymorphs of γ- and the two polymorphs of δ-hexachlorocyclohexane.*

points of the polymorphs are easily obtained on the pure compound. The transition temperature and the two eutectic temperatures are easily determined on any mixture of α and δ ranging from 5 to 30% α. The system is therefore a simple-eutectic diagram of α with each polymorph of δ, and the δ-isomer has two polymorphs enantiotropic with respect to each other. Not all binary systems are so simple. Figure 15, for example, shows the relationship between the three forms of γ-hexachlorocyclohexane and the two forms of the δ-isomer. The different polymorphs in a given system may form isomorphous solid solutions, eutectics, or molecular addition compounds. In fact, different pairs of polymorphs may show different types of composition diagrams. For example, 1,3,5-trinitrobenzene (I) forms an eutectic with picric acid (I), but forms a continuous series of isomorphous solid solutions with picric acid (II). Composition diagrams in organic systems can be as complex as the familiar diagrams for metals and inorganic silicates. Benzalaniline and dibenzyl (71) form an unusual diagram in which the two metastable polymorphs of each compound form a continuous series of solid solutions; yet, over a range of composition from about 35 to 65% dibenzyl, the solid solutions are the stable phase (Fig. 16).

When the binary system shows an addition compound it is extremely unlikely that any pair of polymorphs in that system will show any other type of diagram. An addition compound can itself show polymorphs as in the system, 1,3,5-trinitrobenzene and phenanthrene (72) (Fig. 17).

Determination of Transition Temperatures—One of

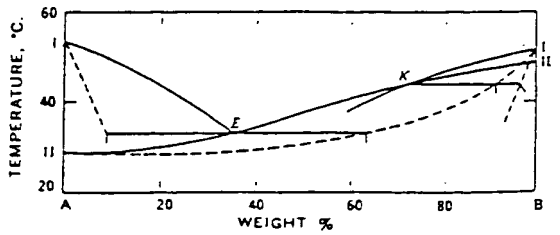

Figure 16—*Composition diagram between benzalaniline (A) and dibenzyl (B) showing stabilization of a metastable lattice by solid solution formation in the range from E to K.*

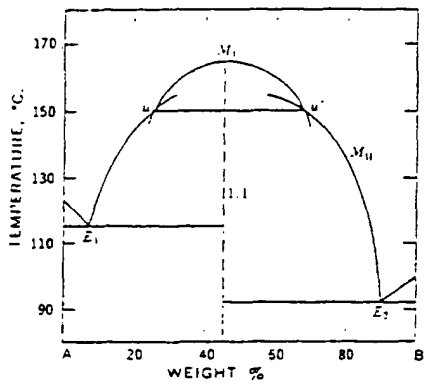

Figure 17—*Composition diagram for 1,3,5-trinitrobenzene (A) and phenanthrene (B) showing polymorphism of the addition compound.*

the most important problems in studying polymorphism in a given system is to determine the transition temperature (70). This not only helps to characterize the system but it also tells which form is stable at low temperatures and when the system is enantiotropic. The transition temperature is the temperature at which the two polymorphs have identical free energies, and hence, at that temperature, both forms have identical solubilities in any solvent as well as identical vapor pressures. The latter are indicated by the *P-T* diagram in Fig. 10. A corresponding diagram can also be drawn, based on the solubility curves (Fig. 18). The transition temperature is best determined by observing a solution phase transformation and noting the temperature at which both forms have the same solubility.

Solution Phase Transformations—The easy way to determine which of two forms is stable at a given temperature is to observe the relative solubility of the two in a solvent. This is best and most rapidly done by observing crystals of both together in a drop of saturated

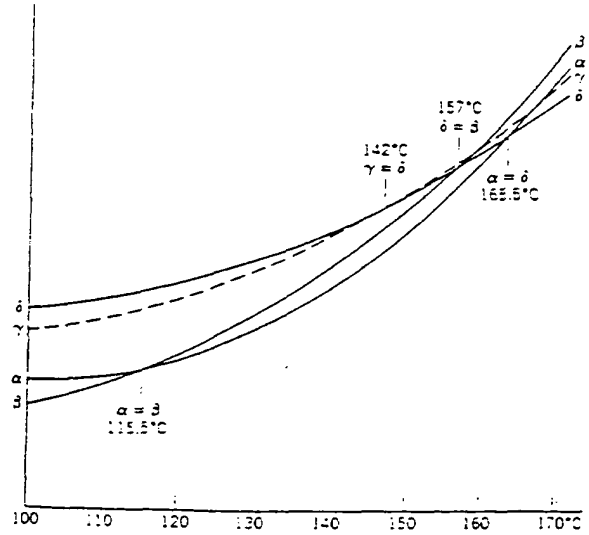

Figure 18—*Solubility curves for the four polymorphic forms of HMX. Again the curves are qualitative, although the intersections are based on the measured transition temperatures.*

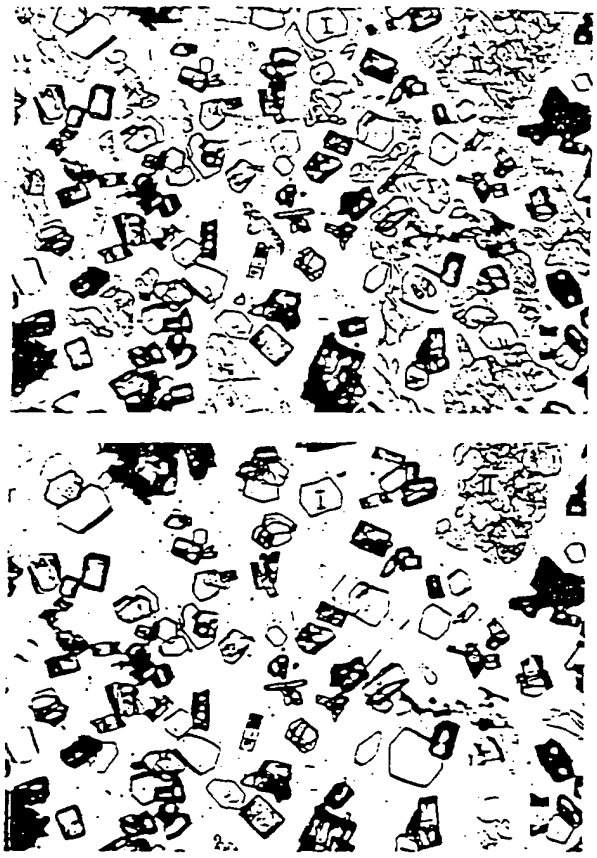

Figure 19—*Photomicrographs of the same field of 1,3-dinitronaphthalene during a solution phase transformation in thymol on a microscope slide. The lower picture was taken about 20 min. after the upper picture.*

solution under the microscope. The less soluble form will grow and the more soluble will dissolve (Fig. 19). This is called a solution phase transformation, and it progresses more rapidly the higher the solubility and the greater the difference in solubility of the two forms. The transition temperature is then the temperature at which the solubilities are equal and at which the transformation rate in either direction is zero. Above the transition temperature one form grows at the expense of the other, and below that temperature the reverse is true. By approaching the transition from both sides, the exact temperature can usually be determined to within a few tenths of a degree. This can often be done using a hot-stage microscope if equilibrium is established rapidly. In some systems it is more convenient to use an apparatus which was designed and used to determine the transition temperatures for the polymorphic forms of HMX (73). A saturated solution containing excess solid is agitated in a narrow flat-bottomed tube, itself immersed in a carefully controlled constant-temperature bath (Figs. 20 and 21). The stable crystal phase existing at a given temperature can be quickly checked by stopping the agitator in the long flat-bottomed tube, allowing the crystals to settle to the bottom and observing them microscopically with the inverted microscope. Figure 22

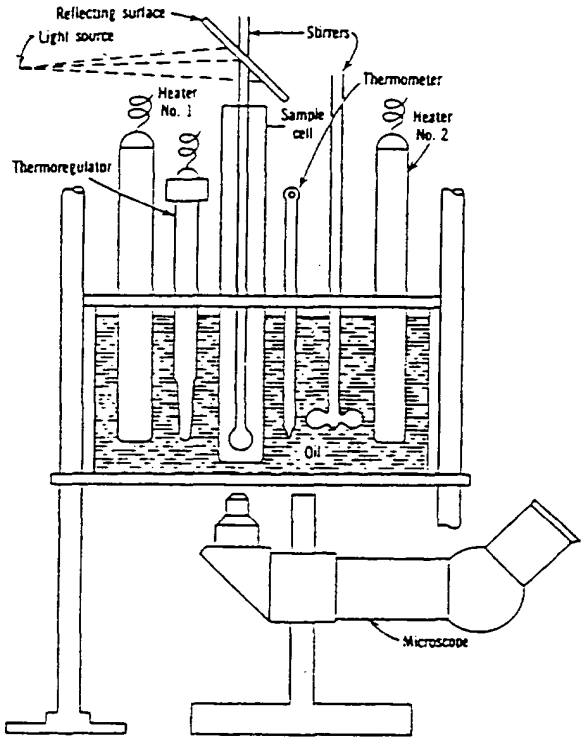

Figure 20—*Experimental arrangement for observing solution phase transformations without disrupting experiment or cooling the sample before examination.*

shows a photomicrograph taken through the inverted microscope shown in Fig. 20 during an experimental run in which a mixture of HMX (I) and HMX (II) had been agitated for several hours at 110°. The needles of HMX (II) are gradually dissolving, showing that HMX (I) (which is growing) is the stable phase at that temperature.

(*a*) Solution Phase Transformation Rates—the rate of the solution phase transformation in a given system depends on the solubility of each polymorph at that temperature, the rates of solution, and the diffusion rate of the molecules in solution. The rate is higher the higher the solubility and the greater the difference in solubilities. The overall rate, of course, is zero at the transition temperature where the solubilities of the two forms are equal, but the rate increases both as the temperature rises and as it falls. However, as the temperature falls further, two factors cause the rate of transformation to decrease again: first, the solubilities of both forms decrease, thus lowering the concentration in solution; and second, the viscosity of the solution increases. Both factors decrease the diffusion rate of the molecules in solution and lower the rate of transformation. The diffusion rate and therefore the transformation rate can also be increased by agitation of the suspension, by using finely divided crystals, and by having seed crystals of the stable form present.

Solid-Solid Transformations—It is sometimes possible to determine the transition temperature by observing only the solid phase during heating and cooling

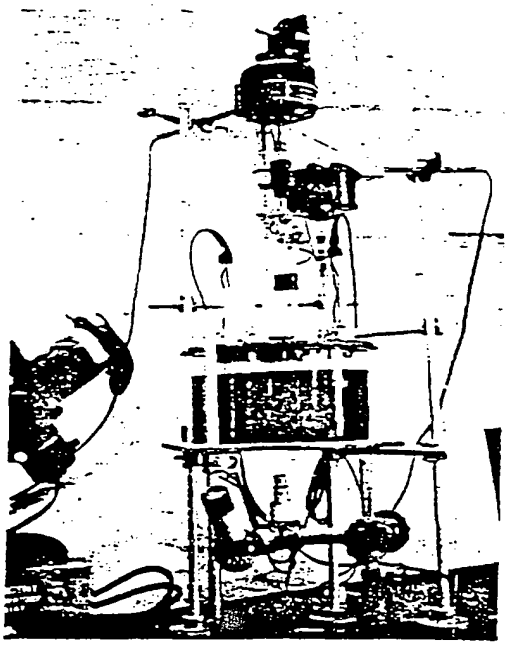

Figure 21—*Photograph of apparatus shown in Fig. 20.*

in the microscope hot stage. It is necessary, however, in this case to repeat the determination several times, and during both heating and cooling, since the transition usually superheats and supercools at least 1° and often many degrees. The solid-solid transformation of HMX (I) — HMX (IV) always occurs in the range 175 to 190° on heating and does not usually reverse on cooling. (The transition temperature, measured easily by observing the solution phase transformation, is 165.5°). Occasionally, superheating and supercooling of the solid-solid transformation may be avoided by holding a preparation containing both polymorphs in physical contact with each other at a series of temperatures on both sides of the transition temperature and

Figure 22—*Photomicrograph of needles of HMX (II) transforming in butyrolactone at 110° to HMX (I). Photomicrograph taken with polaroid camera through the inverted microscope shown in Figs. 20 and 21.*

Figure 23—*A solid-solid polymorphic transformation taking place in a thin crystalline film of canillin. The darker area (pseudomorph) is an area originally unstable and now converted to the stable form.*

observing the direction of movement of the interface between the two forms. This is best done in a thin crystalline film prepared by crystallization from the melt (Fig. 23). The transition temperature of mercuric iodide (128°) can be measured in this way. In passing, it is interesting to note that the transformation rate for HgI_2 and for most other systems (referring only to the solid-solid and not to the solution phase transformation) is much more rapid the second time than the first. In other words, a given crystalline film may both nucleate and transform slowly, but, once a given area has undergone a solid-solid transformation, that region can be transformed more readily back and forth. This is presumably true for displacive rather than reconstructive transformations, since the latter are always quite sluggish and the transition temperatures for such systems must usually be measured by solution phase transformation.

The solid-solid transformation as carried out above can be converted to the always more rapid solution phase transformation by the simple expedient of touching a tiny drop of a low-boiling solvent to the edge of the preparation. Capillarity will draw the solvent into the interstices between the crystals and particularly along the interface between the two forms. The rate of the transformation can be considerably increased in this way if the solvent is a good one for the crystals and if the solution thus formed is not too viscous. (This is the real reason for use of a low-boiling solvent.)

A transition temperature lying below room temperature is more difficult to measure, although with a cold stage for the microscope the same techniques can be used down to about −186°. The crystals are examined microscopically for evidence of pseudomorphs. A pseudomorph is a transformed crystal: the external shape of the original crystal may be discernible even though the internal structure is that of the new form. The pseudomorph may be more or less broken up by the transition if the density difference between the two forms is very great (Fig. 24). Occasionally the pseudomorph may crumble to a dust, even with gentle handling. This phenomenon can sometimes be turned to advantage as a substitute for grinding a substance to achieve small particle size; for example, calcium silicate can be effectively reduced to very fine particles by passing it through the transition temperature.

Although the microscopical procedures for determination of the transition temperature work best between −186° and about 2400°, there are admittedly other ways of detecting transitions. For example, dilatometry may be used to detect the density change on transformation, X-ray diffraction will detect phase changes due to transformation, and, of course, the solubility or vapor pressure curves can be measured for the two forms in order to find the crossover temperature (the transition temperature). Special methods may be used in certain cases; for example, with metals. If an electrolytic cell can be set with the two different forms acting as the two electrodes, there will be a measurable E.M.F at all temperatures except the transition temperature. In monotropic systems, the transition temperature lies above the melting point of both polymorphs. Hence it cannot be located directly, although Schenk (74) has used the following relationship to calculate it:

$$T_t = (F_1K_2 - F_2K_1)/(K_1 - K_2)$$

where F_1 and F_2 are the two melting points and K_1 and K_2 are the molar freezing point depression constants for the two forms. It may also be possible to locate the transition temperature in a monotropic system by measuring the solubility curves or the vapor pressure curves over a range just below the melting points, and extrapolating the curves to their intersection.

Preparation of Metastable Polymorphs—*Methods Based on Thermodynamic Equilibrium*—There are two general methods for the crystallization of metastable polymorphs. The first, based on thermodynamic equilibrium, involves holding crystals of one form in the temperature stability range of the desired polymorph until transformation occurs. If the crystals are wetted with a solvent so that a solution phase transformation can occur, the change will be much faster; if seeds of the desired polymorph can be present, the transformation will be even more rapid. The procedure and apparatus for this are not very different from those de-

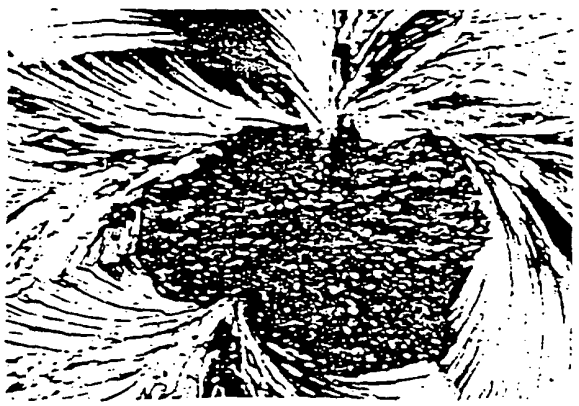

Figure 24—*TNT preparation in which the very unstable form II started to crystallize but was almost immediately transformed to form I which nucleated almost simultaneously on the advancing crystal front of II. The remaining melt then crystallized rapidly as form I.*

scribed above for the determination of the transition temperature (Figs. 20 and 21). It is a help to know the transition temperature, so that the solution phase transformation can be carried out as far as possible from the transition temperature but still in the stability range of the desired polymorph. For example, one can convert HMX (I) to HMX (II) more rapidly at temperatures from 160 to 165°, whereas HMX (II) would best be prepared from HMX (IV) at about 120° and from HMX (I) at about 160° [HMX (I) ⇌ HMX (II) = 115.5°, HMX (II) ⇌ HMX (IV) = 165.5°]; see Figs. 12 and 13 (70). This method of preparing a high-temperature polymorph works, of course, only in enantiotropic systems in which the desired form has a definite temperature stability range.

Methods Based on Prevention of Thermodynamic Equilibrium—In monotropic systems it is usually relatively easy to prepare the metastable forms, but it is necessary in so doing to prevent thermodynamic equilibrium by working fast and with small quantities (67). In general, a metastable polymorph (in enantiotropic systems as well as monotropic systems) can be prepared either by achieving a high degree of supersaturation in the vapor or solution state, or by supercooling in the melt state. The general objective is to find conditions under which the metastable form can crystallize before crystals of the stable form appear. Since the metastable form will always be more soluble and more volatile and will have the lower melting point, it follows that supercooling is necessary in order to crystallize the metastable form from the vapor, melt, or solution. The system must then be supercooled below the melting point of the metastable form, and at the same time one must prevent crystallization of the more stable form or forms. With HMX, for example, it is possible to prepare any one of the four crystal forms from the same solvent and at temperatures all well within the thermodynamic stability range of HMX (I). This is done by supercooling the solution rapidly. The faster the cooling, the more unstable will the polymorph be. The rate of cooling is most readily controlled by varying the volume of the solution, since a small volume can be cooled very rapidly.

Solution Phase Preparations of Metastable Phases—The various polymorphic forms of HMX are usually obtained by starting with an acetone solution saturated with HMX (I) at 63° and with no excess solid present. To obtain HMX (I), 200 ml. of this hot solution is cooled spontaneously to room temperature. This gives very slow cooling, lower supersaturation, and crystallization of pure HMX (I). To obtain HMX (II), 50 ml. of the same hot solution is cooled to room temperature with gentle agitation (swirling, to improve heat transfer). HMX (III) results if 30 ml. of the same hot solution is cooled with agitation in an ice-water bath. HMX (IV) is formed when 5 ml. of the solution is cooled in a test tube by immersion (with shaking) in a dry-ice cooling bath or by pouring 5 ml. of the hot solution over cracked ice. In every case, the crystals must be filtered off rapidly to avoid solution phase transformation to HMX (I) which is stable up to 115.5°. Other solvents may also be used; however, the cooling rates must be changed in accordance with the solubility of HMX in that solvent. If HMX is more soluble than in acetone, the rates of cooling must be increased; if less soluble, the rates must be decreased.

There are three reasons for this relationship between solubility and cooling rates, although all are based on the fact that the concentration of the compound is less in the poorer solvent. First, in such a solution it is more difficult to nucleate any solid phase because nucleation is a 2-, 3-, or 4-body collision process and there is less probability of nucleation in a dilute solution. The necessary degree of supercooling can, therefore, be achieved. Second, the high-temperature (low-density, more symmetrical, and more open) structures nucleate most readily when they can (sufficient supercooling). This is the basis for Ostwald's law of successive states, which says that a phase change will occur step by step by the way of successively more stable phases. Third, and finally, in a poor solvent the rate of transformation of a metastable to a more stable polymorph is slower. Hence a metastable form once crystallized can be isolated and dried before it is converted by solution phase transformation to a more stable phase.

In some systems the metastable form is extremely unstable and may be prepared only with more extreme supercooling. This is usually done on a very small scale (even a single drop of solution) with high-boiling liquids, so that a saturated solution at a high temperature suddenly cooled to room temperature will achieve a high degree of supersaturation. If, for example, a saturated solution of RDX (cyclotrimethylene trinitramine), HND (hexanitrodiphenylamine), or ammonium picrate in a drop of thymol on a microscope slide at 150–200° is cooled rapidly by placing it on a cold surface, crystals of the corresponding unstable polymorphs will appear (67). In each case, some crystals of the stable form may also appear, and a slow solution phase transformation may occur. The rate of this transformation will increase with increasing temperature.

Preparation of Metastable Phases from the Melt—An analogous behavior results when a melt rather than a solution is supercooled. One of the most interesting systems studied thus far (67) is DINA [bis(β-nitroxyethyl)-nitramine]. This compound shows four crystalline polymorphs, each of which can be prepared easily from the melt and each of which has a measurable melting point. The melting points are: DINA (I), 52°; (II), 52°; (III), 38°; and (IV), 30°. DINA (I) melts about 0.05° higher than DINA (II). A completely melted sample of DINA supercools to room temperature and remains indefinitely without crystallizing. If, at any temperature below 52°, the glass cover slip is pressed hard into contact with the microscope slide, using the side of a teasing needle or even a pencil eraser, nucleation usually occurs at the point of contact. The temperature of the preparation at the time of crystallization determines the degree of supercooling and therefore the polymorphic form. Below 30°, DINA (IV) results; at 30–35°, DINA (III); at 40 to 45°, DINA (II) is the usual product, although DINA (I) or a mixture of I and II may appear. Each of these forms is reasonably stable on a microscope slide, although IV, as would be expected, is least stable. DINA (III) usually spontaneously nucleates in Form IV within a few minutes.

Some compounds show an unstable short-lived polymorph when the melt is highly supercooled. Good examples are acetanilide and TNT. Both should be melted completely and allowed to cool quietly and undisturbed. Within a few minutes for acetanilide, or a few hours for TNT, crystallization will occur, but the unstable form will immediately and very rapidly transform to the stable form, leaving an obvious pseudomorph (Fig. 24). As these few examples show, it is not difficult to form a sound idea of the relative stability of the unstable forms of different compounds. The TNT polymorph is probably as unstable as any ever observed, since it always transforms to the stable form, probably in a few hundredths of a second after it forms. It is necessary to supercool a number of tiny droplets less than 1 to 2 mm. in diameter to observe the TNT pseudomorphs, and even then the percentage of crystallizations showing the phenomenon is less than 1%. Acetanilide (II) is somewhat more stable, forming a fair percentage of the time from 2 to 3 mg. of melt and with an average life of perhaps a second. Again, it is obvious as a pseudomorph. The DINA polymorphs are much more stable than those of either TNT or acetanilide. There is, of course, no possibility of obtaining TNT (II), acetanilide (II), DINA (III) or (IV), RDX (II), HND (II), ammonium picrate (II), or HMX (IV) on a macro scale. Another interesting example of a highly unstable polymorph is picric acid. Form I melts at 121°, and Form II melts at 75°. It is understandably difficult to supercool picric acid 46° without getting crystals of Form I, but it can be done in very small preparations (0.1 mg.) between a slide and cover slip. It is then necessary to cause form II to crystallize; this has been done by seeding with trinitrobenzene (I), with which picric acid (II) is isomorphous. This is obviously best done in a fusion preparation by recrystallizing TNB as the stable Form I, then melting a very tiny amount of picric acid at the edge of the cover slip near the TNB so that it is absorbed (so to speak) by the TNB melt and forms a small pocket of picric acid surrounded on three sides by TNB. On rewarming, the picric acid and a portion of the TNB are melted, and, on cooling, the unmelted TNB (I) seeds the melt including the picric acid. The rate of crystallization is very rapid through the TNB (I) but slows considerably in the zone of mixing, then speeds up slightly in the picric acid (II). The latter may remain for a few minutes or a few seconds, but it always transforms very soon into picric acid (I) pseudomorphs of (II).

In general, it becomes more difficult to obtain the room temperature stable form as the transition temperature decreases to within 10 to 20° of room temperature. The compound 1,2,5-trinitronaphthalene melts at 112 to 113° (II) but has a transition temperature at about 30°. As a result the stable Form I cannot be obtained without experiments below room temperature (or 20°). If the transition temperature falls below room temperature, the low-temperature form will not usually be observed unless experiments are carried out at lower temperatures within the required temperature range. It is significant that a given polymorphic form can often be crystallized well below its temperature stability range, but seldom, if ever, above it. Presumably this is due to the fact that a supercooled system will lack the lattice mobility to transform; hence an unstable polymorph well below the transition temperature may be very stable once it is formed. This is useful in telling us that in those cases of great instability of one polymorph, *e.g.*, acetanilide, RDX, HND, the system is probably monotropic.

Preparation of Metastable Phases from the Vapor—Preparation of metastable forms by sublimation is a useful procedure, especially because the individual small crystals of the metastable forms are quite stable and can be used directly for melting point determinations (5). The general procedure utilizes a Kofler sublimation block and a temperature at which the compound sublimes readily. The temperature of the cover slip then determines the crystal form and size of the sublimate. A very small temperature difference between the source and the condensing surface will result in condensation of the stable form at that temperature. Cooling of the cover slip by placing drops of various low-boiling solvents on the top surface will cause condensation of the more unstable forms, the lower temperatures leading to the most unstable forms. A vapor phase transformation may, of course, occur if two different crystal forms condense, since each has a different vapor pressure (Fig. 25).

Stability, Metastability, and Instability—The idea that unstable crystal forms can be readily prepared at temperatures far below their normal temperature stability ranges is one of the unappreciated ideas in the study and handling of polymorphs. It is not inconceivable that diamonds, now being produced by attaining thermodynamic stability, *i.e.*, high temperatures and pressures, might some day be made at ordinary pressures and quite low temperatures. The requirement, and for carbon a tough one, is a liquid solvent at temperatures below the range at which diamond changes to graphite by a solid-solid transformation.

Diamond, of course, is unstable thermodynamically at ordinary temperatures and pressures. It is said to be

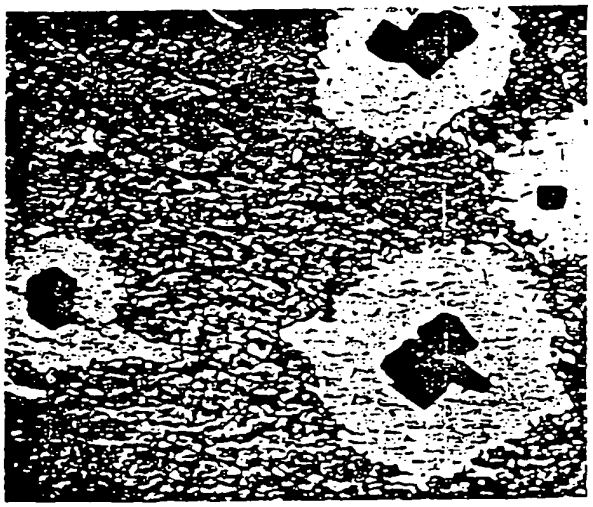

Figure 25—*Sublimate of two polymorphic forms of picramic acid. The unstable form has vaporized in the neighborhood of (and onto) the stable form.*

metastable, and many such materials are known. Many minerals (aragonite, anatase, brookite, etc.), many drugs (75) (atophane, progesterone, estrone, marfanil, sulfathiazole, veronal, *etc.*), and even some important metals (copper, silver, zinc, tin, bismuth, and cadmium) are used daily in the metastable form. The degree of stability of a metastable polymorph varies considerably. Diamond is one extreme, although even diamonds often transform, on heating, to graphite. At the other extreme is TNT, which possesses a metastable form so short-lived that no one has even seen it. Only the pseudomorph remains to tell us that a transformation has occurred (Figs. 23 and 24). The degree of metastability in a given system can be changed to some extent. For example, crystal size is a definite factor, and large crystals of a metastable form are usually much less stable than small crystals. Large crystals of HMX (IV), 1 mm. or more in large dimension, seldom last longer than a few hours before they transform to HMX (I). On the other hand, very small crystals of HMX (IV) less than 50 μm. long and 10 μm. thick, prepared in 1942, are still unchanged more than 25 years later. Physical shock, especially grinding, is usually an effective way to convert a metastable crystal form to the stable form. Presumably this conversion occurs most readily for displacive-type transformations (76), since there are many metastable systems, such as diamond, that can be pounded to angstrom dimensions without transformation. Grinding diamond at elevated temperatures would undoubtedly increase the rate of solid-solid transformation to graphite.

Pseudopolymorphism—Pseudopolymorphism is a convenient term to use to describe a variety of phenomena sometimes confused with polymorphism. They include desolvation, second-order transitions (some of which are polymorphism), dynamic isomerism, mesomorphism, grain growth, boundary migration (77) recrystallization in the solid state, and lattice strain effects.

Of these, probably the most important is desolvation. During heating of a crystalline solid in the microscope hot stage, one evidence of polymorphism is a solid-solid transformation. This results in darkening of the crystal, observed by transmitted light. It is due to the breaking up of the crystals (the density change is often 1% or more) and light scattering by the many new air-crystal interfaces. This change is, in visual detail, duplicated when a crystalline solvate gives off solvent of crystallization on heating. There is, however, a simple microscopical procedure to differentiate polymorphism from desolvation. The heating experiment is repeated with the crystals completely immersed under a cover slip in a liquid immiscible with the possible solvent of crystallization. If, then, desolvation is involved, heat will produce the desolvated solid plus a liquid droplet of the solvent. If polymorphism is involved, the change will occur as before but no liquid phase will appear until the final melting point.

Study of a Compound to Determine whether Polymorphism Exists—It is comparatively easy to determine by microscopical procedures whether a given compound can crystallize in two or more different crystalline polymorphs. The following are standard procedures designed to cause crystallization of the metastable form.

(*a*) Melt completely a small amount of the compound between a slide and cover slip and observe the solidification between crossed polars. If, after spontaneous freezing, a solid-solid transformation occurs spontaneously or can be induced by seeding or scratching, the compound exists in at least two polymorphic forms. The problem, in this case, is to prevent crystallization of the stable form by inducing supercooling. Four steps can be taken to help induce supercooling: First, the sample size should be very small: in fact, a number of separate droplets under the cover glass, ranging in diameter from a few tenths of a mm to 1 to 2 mm., will provide the most satisfactory size. Second, the crystals should be melted completely by holding the melt for 30 seconds or so about 10 to 20° above the melting point. Third, the melted preparation should be carefully set aside for several minutes without physical shock before examination. Only under these conditions can the very unstable form of TNT be obtained. To a slightly lesser extent, formation of the metastable form of acetanilide requires the same care. Even in spite of these precautions, the metastable polymorphs of these two compounds are seldom observed: only the pseudomorph remains as evidence of the formation of the metastable form. Finally, one should try rapid cooling of the sample using one of the aerosol freezing jets directed against the cover slip preparation. These cans of fluorocarbon refrigerant (Freon) are available as martini glass chillers.

Pentaerythrityltetranitrate (PETN), carbon tetrabromide, mercuric iodide, ammonium nitrate, and 1, 3,5-trinitrobenzene (TNB) always crystallize on cooling to give the high-temperature form if they are completely fused. Of this group, PETN, HgI_2, CBr_4, and ammonium nitrate show spontaneous transformations on cooling, but trinitrobenzene must usually be scratched or seeded to induce a transformation.

It is often of value to carry out a meltback (partial remelting) and seed the melt with some of the compound from the stock bottle (presumably, though not always, the stable form). As freezing proceeds, the junction of the two crystal fronts is observed for evidence of polymorphism. In general, if the two crystal fronts are made up of different crystalline modifications, one of them will grow into and through the other on contact.

(*b*) Heat a sample of the compound in a hot stage and observe whether a solid-solid transformation occurs during heating. A transformation will occur with most compounds which show enantiotropic transformations, *e.g.*, ammonium nitrate, carbon tetrabromide, and ammonium picrate. Many other systems, even though enantiotropic, do not readily transform under these conditions. The changes are greater the larger the preparation: hence 10 to 20 mg. should be used.

(*c*) Sublime a small quantity of the compound and attempt to induce a solution-phase transformation between the sublimate and the original sample by mixing the two in a drop of saturated solution of one of them. If the two are polymorphs, the more stable will be more insoluble and will grow at the expense of the more soluble metastable form. This process will continue at a rate which depends on the difference in solubility and the absolute solubility until the metastable form is com-

pletely transformed to the stable form. If the two samples are not polymorphs but are different compounds, one may dissolve but the other will not grow (except perhaps as the solution evaporates). If the two are identical forms of the same compound, no change will occur on standing in solution. (Remember that the solution in use is, at the beginning, a saturated solution of either one of the two samples.) This method works only for systems in which the compound condenses on sublimation as a polymorph different from the original. To increase this possibility, different condensing surface temperatures should be tried. A low-condensing surface temperature favors the formation of a metastable form.

(*d*) Maintain an excess of the solid compound in a small amount of solvent held at a temperature as near the melting point of the compound as possible. Hold for several hours, then isolate the suspended solid by decantation followed by drying at the refluxing temperature. Any method of quickly isolating these crystals or, at least, keeping the temperature from dropping during the entire operation is satisfactory. Test the product with a sample of the original compound for solution phase transformation as outlined above under (*c*). This procedure works for any enantiotropic system, *e.g.*, styphnic acid, HMX, HgI_2, in which the material can be maintained in the temperature range of the high-temperature form for a sufficiently long time for the solution phase transformation to occur. Using a solvent boiling in the desired temperature range solves the problems of temperature control and of agitation.

(*e*) Recrystallize the compound from a small amount of solution by cooling very rapidly (pour onto ice, or drop dropwise on a chilled microscope slide, *etc.*), and observe a portion of the precipitate suspended in a drop of the mother liquor. The drop may then be seeded with the original compound to check for solution phase transformation. If the precipitate is a different polymorph, a solution phase transformation should take place. This method of obtaining polymorphs has been used successfully for hydroquinone and for HMX.

A very useful modification of this test involves the use of high-boiling solvents, such as butyrolactone, thymol, benzyl alcohol, and nitrobenzene. If these compounds are used, it is possible to carry out the recrystallization in a drop on a microscope slide. Under these conditions cooling will be very rapid, and it is possible to obtain high-temperature polymorphic forms that have not been prepared by any other method. For example, RDX and HND were found to exist in at least two polymorphic forms by recrystallizing from thymol after all other methods of detecting the presence of polymorphs had failed.

Are Two Given Samples Polymorphic Forms of the Same Compound?—Another general problem is the recognition of polymorphism as the relationship between two different crystalline solids. Some general procedures for achieving this are given below. Because many of the phenomena listed above (tautomerism, polymerism, strain, *etc.*) can cause a system to behave as though it is polymorphic, it is necessary to eliminate these possibilities before concluding in any given case that polymorphism is present. In general, if the two phases are different crystallographically (as determined by X-ray diffraction or petrographically), then crystal strain can be eliminated. If the two crystal forms give identical melts or solutions at the instant of melting or dissolving, then tautomerism and polymerism are eliminated. If the two crystal forms are solid (*i.e.*, do not flow with pressure on the cover slip), then mesomorphism is eliminated. In the entire list of procedures to be given below it is assumed that grain growth due to strain has been eliminated.

1. If two samples have different crystal properties, *i.e.*, axial ratios, refractive indices, densities, and X-ray powder patterns, and can be converted into each other through a solid phase or a solution phase transformation, they are polymorphic forms of the same compound.

2. If the two compounds are different in all crystal properties, as in (1.), yet melt to give a liquid having the same refractive index of the melt and the same temperature coefficient of refractive index, the two samples are likely to be polymorphs of the same compound. If the two solids are mixed and heated to the first melting point and held there, a pair of polymorphs will either crystallize completely as the high-melting stable form or will melt completely (if both forms melt very close together). If the system remains as a mixture of solid and melt at that temperature, then the two solids are different compounds.

3. If a mixed fusion between the two samples shows identity, yet the crystal properties are different, as in (1.), then the two samples are polymorphs of the same compound.

4. If one of the samples is fused and allowed to supercool slightly below the melting point, the melt can be seeded at different points with each of the solid samples. If the resulting solidification is observed using a low-power microscope having crossed polars, observation at the junction of the two solids will indicate the presence or absence of polymorphism. If, at the junction, one of the two forms continues to grow through the other solid, the two forms are polymorphs. If the two solids grow together with no subsequent change, even on rewarming and long standing, then the two samples are identical and are not polymorphs (assuming that one of the seed crystals did not transform during the seeding operation). Seeding alone is not, however, proof that the crystal form of the seed material and the growing crystals are the same phase or even, necessarily, the same compound. Seeding with any material involves some physical shock which is often sufficient, by itself, to cause nucleation.

5. If the two samples are mixed dry on a microscope slide beneath a cover slip, they can be wetted by allowing a liquid to run in under the edge of the cover slip. If they are wetted with a saturated solution of either component in a suitable solvent, the occurrence of a solution phase transformation means that the two samples are polymorphic forms of the same compound.

6. If several crystals of each sample are heated close together in a hot stage, a solid-solid transformation of one component followed by melting of both at the same temperature will strongly indicate (but not prove) that the two are polymorphs. If, on heating, one form melts first, then resolidifies completely owing to seeding

by the other form, followed by uniform melting on further heating, the two forms are polymorphs.

7. There might also be a vapor phase transformation during heating of the two samples in intimate mixture. This too is characteristic of polymorphism.

Conclusions—With the foregoing discussion, it is clear that probably every organic medicinal can exist in different polymorphs and the choice of the proper polymorph will determine if a pharmaceutical preparation will be chemically or physically stable, or if a powder will tablet or not tablet well, or if the blood level obtained will be the therapeutic level to give the pharmacologic response desired. Thus, it is time that pharmaceutical companies, as a part of their preformulation studies, identify and study the stability of different polymorphs of each potential new drug, as they do the melting points or other physical characteristics.

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